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CONTRACTING ORGANIZATION: University of Texas Health Science Center at San Antonio
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The objective of this program is to establish at the University of Texas Health Science Center in San Antonio an in-depth training program in the Molecular Genetics of Breast Cancer. The most important goal of the program is to train highly qualified pre-doctoral students in the genetic, cellular, and molecular basis of Breast Cancer. It is our hope that with the background in Breast Cancer Biology that these students have obtained, they will complete their studies and provide the momentum and scientific expertise for significant discoveries in this field in the future.

The program has a number of major strengths. These include the high quality of the Program Faculty, the interactive nature of the Breast Cancer research community in San Antonio. The Training Program in the Molecular Genetics of Breast Cancer has been extremely successful in attracting high quality pre-doctoral students. This progress report reviews these accomplishments.

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FOREWORD

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PI - Signature

10/9/95
Date

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1. Brief Description of the Training Objectives and Goals

The objective of the program is to establish at the University of Texas Health Science Center in San Antonio an in-depth training program in the Molecular Genetics of Breast Cancer. The most important goal of the program is to train highly qualified pre-doctoral students in the genetic, cellular, and molecular basis of Breast Cancer. It is our hope that with the background in Breast Cancer Biology that these students have obtained, they will complete their studies and provide the momentum and scientific expertise for significant discoveries in this field in the future.

The issues raised in the second criticism of the lettered dated 01/11/96 are the result of relatively narrow interpretations of what constitutes direct or peripheral research in breast cancer. A strength of the breast cancer research in the Molecular Medicine program revolves around breast cancer genetics. Thus, basic training in genetics is a necessary prerequisite for application to breast cancer. Another point that we would like to stress in this letter is that breast cancer cells are not always the best cells in which to study genetic and cellular mechanisms involved in breast cell tumorigenesis. Once the mechanisms are elucidated, the hope is to then apply that knowledge to understanding breast cancer cells and, more importantly, to tumorigenesis in animal breast tissue. However, on the whole, we believe that most of the students supported by the breast cancer training program have been and are being trained in breast cancer research.

Christa Hargrave and Harold Pestana, two entrance level students supported by the Breast Cancer Program in 1994-95, were, during this period, taking basic courses in molecular biology, molecular genetics and cell biology. During his final laboratory rotation, Harold Pestana worked on a project in Dr. Dave Sharp's laboratory involving Pit-1, a regulator of prolactin and growth gene expression. Recently, evidence from Dr. Sharp's laboratory and others has been obtained showing an autocrine growth pathway involving prolactin and prolactin receptor in the self-stimulation of the growth of breast cancer cells. Thus, Harold's project on Pit-1 and prolactin gene regulation is directly related to breast cancer.

Zachary Mackey's first project was in Dr. Kent Osborne's laboratory where he worked on tamoxifen resistance and breast cancer. His current project in Dr. Alan Tomkinson's lab is DNA ligase I and its role in DNA-repair, which is also directly related to breast cancer. DNA damage in breast cells may result in genetic disruptions at an early time in tumorigenesis. The fact that DNA damage and repair are directly related to all cancer doesn't negate its importance in breast cancer.

Jim Fitzgerald's project on the regulation of adipose-specific gene expression does at first glance appear to be peripheral to breast cancer research. However, it is known that the lipogenic gene products Mr. Fitzgerald is studying are up-regulated in fat cells of the breast which then produce growth factors and/or lipids that promote cellular proliferation and transformation. This is a good example of what may appear to be research tangential to breast cancer turning out to be directly related. We apologize for not having made this point clearly in the progress report.

Yuewei Qian's project involving retinoblastoma associated proteins is also directly related to breast cancer by virtue of the central role of Rb in the cell cycle and tumorigenesis. Again, the universality of Rb's role in cell cycle and tumor suppression does not negate the potential importance it has in breast tumorigenesis.

Yi-Chun [James] Wang is the only student whose work might be considered peripheral to breast cancer. Having recognized this, the Directors removed him from the program and he is now supported by his mentor's funding. Originally it was thought that viral latency and tumorigenesis by EBV might be educational in terms of breast cancer, but since no role for EBV has been established in mammary tumorigenesis, James was taken out of the program.

The four entering students in 1995-96 supported by the program are in the process of deciding about the direction of their research careers. Linda deGraffenried's first laboratory rotation was in Dr. Sharp's laboratory where she was instrumental in showing prolactin expression in human MCF-7 breast cancer cells. She is currently in Dr. Kent Osborne's laboratory where she is pursuing a novel project on the role of haptoglobin as a growth factor for breast cancer cells. Her next rotation will be with Dr. Suzanne Fuqua where she will, in collaboration with Dr. Sharp, study the role of the estrogen receptor in prolactin-mediated growth stimulation of breast cancer cells. It should be noted, that Ms. deGraffenried entered the Molecular Medicine program because of the strong programs in breast cancer research. It is this type of student that the Molecular Medicine Program in conjunction with the Breast Cancer Training Program will be aggressively recruiting in the future.

Jennifer Gooch also did her first rotation in Dr. Sharp's laboratory where she did a Pit-1 mutagenesis project. She became interested in breast cancer research and chose as her second rotation, Dr. Douglas Yee's laboratory where she is currently working on signal transduction in breast cancer cells.

David Levin's first rotation was in Dr. Alan Tomkinson's laboratory where he did a project on DNA repair. His second rotation is in the laboratory of Dr. Jolene Windle. Dr. Windle is an expert in the development of animal models of tumorigenesis and cancer research.

Ernest Salcedo's first rotation was in Dr. Paul Gardner's laboratory where he worked on the genetic regulation of the receptors involved in neurotransmission. His second rotation is in the laboratory of Dr. Steve Britt where is he is interested in learning the fundamentals of genetics using the fruit fly, Drosophila melanogaster.

From the foregoing, it is clear that most students supported by the breast cancer training program are, indeed, engaged in research directly related to the problem of breast cancer. Two additional points need to be stressed. There is, at the current time, very intense nationwide competition for well qualified domestic students. In the last cycle of recruitment, Molecular Medicine was very successful in enrolling four excellent domestic students, one of which came to the program with an interest and intention of working on breast cancer. Each of these new students has indicated an interest in breast cancer research. That brings us to the last point which is that, in the Molecular Medicine Ph.D. Program, graduate students are free to choose the research laboratories and projects on which they want to work. In our new Breast Cancer Training Program, it is, perhaps, unrealistic to think that, in the first year of Army support, that all of the students funded by the Breast Cancer Program will choose mentors and projects directly related to breast cancer. It is anticipated that by the end of the current funding period, the training program will be supporting students who are all working directly on the

problems associated with breast cancer. Clearly, our progress report and this letter demonstrate that we are making excellent progress in that direction.

One of the major strengths of the program is the high quality of the Program faculty, and the interactive nature of the Breast Cancer research community in San Antonio. The program faculty are organized into four subprograms, which encompass scientists and physicians studying different aspects of breast cancer and cancer therapy, as well as fundamental mechanisms of cell growth, differentiation and molecular genetics. These faculty groupings are listed here, detailed descriptions of individual research programs were included in the original application.

A. Breast Cancer Sub-Program

C. Kent Osborne, M.D.
John Chirgwin, Ph.D.
Suzanne Fuqua, Ph.D.
E. Lee, Ph.D.
W.-H. Lee, Ph.D.

B. Growth Factor Sub-Program

Douglas Yee, M.D.
Gregory Mundy, M.D.
Barbara H. Bowman, Ph.D.
Robert J. Klebe, Ph.D.
Betty Sue Masters, Ph.D.

C. Drug Development Sub-Program

Daniel Von Hoff, M.D.

D. Molecular Genetics Sub-Program

Robin Leach, Ph.D.
Peter O'Connell, Ph.D.
W.-H. Lee, Ph.D.
Z. Dave Sharp, Ph.D.
Edward Seto, Ph.D.
Alan E. Tomkinson, Ph.D.

Each of these faculty members maintains an active research program. A listing of their research support is found below.

In this progress report, the relationship between the Breast Cancer Training Program and the Molecular Medicine Graduate Ph.D. Program is reviewed, and additional or updated information is provided regarding:

3. Research Support for Program Faculty
4. Listing of Supported Trainees
5. Project Summaries of upper level trainees
6. Trainee publications
7. Changes to the Program Faculty:
   Additions:
   Alan E. Tomkinson, Ph.D. to Molecular Genetics SubProgram

Biographical Sketch, Research Support, Project Summary

8. Course Changes:
   New Course:
   Current Topics in Cancer Biology / Course Director: Eva Lee Ph.D.

2. Relationship between the Breast Cancer Training Program and the Molecular Medicine Graduate Ph.D. Program

The Breast Cancer Training Program has been implemented within the context of the Molecular Medicine Graduate Ph.D. Program. The Molecular Medicine Ph.D. Program is a recently established interdisciplinary Ph.D. training program in the Graduate School of Biomedical Sciences at the UTHSCSA. The Breast Cancer Training program takes advantage of the internationally recognized breast cancer research program existent in the institution for many years, and offers a unique opportunity for students interested in starting careers in breast cancer research. The participating scientists in this breast cancer program represent diverse departments including the Division of Medical Oncology, Hematology and Endocrinology in the Department of Medicine, and the Departments of Cellular and Structural Biology, Pathology and Biochemistry. In addition, the new University of Texas Institute of Biotechnology and the San Antonio Cancer Institute [SACI], an NIH-designated Cancer Center, represent outstanding resources for training opportunities in clinical and basic science research. The national and international reputation of the participating faculty serve to attract a large number of excellent applicants to the breast cancer research track in the Molecular Medicine program. The awarding of a Breast Cancer Specialized Program of Research Excellent (SPORE) grants to the institution documents the quality of breast cancer research available to trainees.

The rationale for administering the breast cancer training program in the Molecular Medicine Ph.D. program is based on several important criteria: [1] The Molecular Medicine curriculum is specifically designed to provide basic science training while integrating fundamental principles of molecular biology with modern medicine. A molecular medicine Core course provides students with the mechanisms underlying human disease and provides intensive review of specific diseases [including breast cancer] that may serve as models for how human diseases can be studied at the molecular genetic level. [2] The Molecular medicine program requires the participation of both clinical and basic scientists in the training process. The inclusion of MDs on all student advisory committees insures that every graduate has a clear perspective on the clinical relevance of the basic research in their program, that in most instances, will serve as a guide for the project. [3] The Molecular Medicine program is an interdepartmental, interdisciplinary program that offers tremendous flexibility to students in terms of research laboratories, advisors and committee members. This arrangement offers a real potential for synergism in breast cancer research not possible in traditional department-bound programs. In summary, our program offers a near perfect environment for Ph.D. training in breast cancer and has attracted many well-qualified applicants.

3. Research Support for Program Faculty

An essential component of maintaining a successful and aggressive training program in Breast Cancer Research is the continued research funding of the individual Program Faculty laboratories. Current funding for each member of the Program faculty is detailed in Table 1. As can be readily seen from the table, the faculty have been extremely successful in obtaining research funding, including over $18,000,000 in direct costs for the 1995-1996 fiscal year.

OTHER SUPPORT

PARTICIPATING FACULTY MEMBER	FUNDING AGENCY	IDENTIFYING NUMBER AND TITLE	PROJECT PERIOD	CURRENT YEAR DIRECT COSTS
Lee, W.-H. ***Active Support***	Council for Tobacco Research	2992 Characterization of a Novel Cell Death Protein Regulated by Retinoblastoma Protein	07/01/95-06/30/96	76,521
	NIH/NEI	5R01EY05758-12 Molecular Basis of Retinoblastoma Formation	03/01/93-02/28/98	218,460
	NIH/NCI	2R01CA58318-03A1 Cancer Suppression by the Retinoblastoma Gene	05/01/95-04/30/98	157,748
	NIH/NCI	Translational Research in Breast Cancer - Developmental Project Do BRCA1-Deficient Mice Develop Breast Tumors? C. Kent Osborne, P.I.	09/01/95-08/31/96	40,000
	Texas Higher Education Coordinating Board	#3659055 Identification of Novel Molecular Markers for Cancer Progression	01/01/94-12/31/95	80,197
	NCI/NIH	1R01CA58183-04 SPORE in Breast Cancer, Project 5 Tumor Suppressor Genes in Breast Cancer Development C. Kent Osborne, P.I.	09/01/95-08/31/96	180,508
Pending Support	Texas Higher Education Coordinating Board	Mitosin: A Novel Nuclear Protein Important for Mitotic Progression	01/01/96-12/31/97	66,161

Lee, W.-H. ***Pending Support***	USAMRMC	Role of BRCA1 in Breast Tumorigenesis	10/01/96-09/30/00	167,394
Osborne, C.K. ***Active Support***	NIH	1PO1CA58183-03 Translational Research in Breast Cancer--San Antonio	09/30/92-09/29/95	1,176,950
	Cancer Therapy and Research Center	Program Leaders		46,356
	NIH	P01CA30195-15 Medical Oncology Program Project; Therapeutic Research; Project 3; Markers of Evolutionary Stages in Breast Cancer	07/28/92-02/28/97	73,269
	NIH	P01CA30195-15 Medical Oncology Program Project; Therapeutic Research; Project 5; TGF-alpha in the Pathogenesis of Breast Cancer	07/28/92-02/28/97	102,040
	NIH	P01CA30195-13 Medical Oncology Program Project; Therapeutic Research; Project 6; The IGF-System as a Potential Treatment Target in Breast Cancer	07/28/92-02/28/97	125,906
	NIH	1K12CA01723-03 Physician Scientist Training Grant in Oncology/Hematology	09/05/92-08/31/97	186,545
	Celby-Geigy			25,000
Pending Support		none		
Bowman, B.H. ***Active Support***	NIH	2P01AG06872 Program Project, Molecular Genetic Mechanisms of Aging	05/01/91-04/30/96	693,151

Bowman, B.H. ***Active Support***	NIH	SG#1P30CA54174-01 Institute for Cancer Research and Care-Cancer Therapy and Research Center	09/01/91-07/31/96	24,188
	AHAF Research Center	Over-Expression of Human APOE4 in Transgenic Mouse Brains	04/01/94-03/31/96	100,000
	NIH	1P03AG13319-01 Nathan Shock Center of Excellence in Basic Biology of Aging	07/10/95-06/30/00	104,000
Pending Support	NIH	Program Project, Molecular Genetic Mechanisms of Aging	05/01/96-04/30/01	894,077
Chirgwin, J.M. ***Active Support***	NIH	P01CA40035 Mechanisms of Bone Resorption in Breast Cancer; Project 2	02/01/89-05/31/94	57,151
	Veterans Administration	VA Merit Award Molecular Basis of Lysosomal Targeting	03/01/93-02/29/97	79,761
	Veterans Administration	Associate Research Career Scientist	03/03/89-02/29/95	38,700
	NIH	AR39529 The Osteoclast and its Regulation Project 5; Pathobiology of Piaget's Disease	04/01/92-03/31/98	59,900
Pending Support		none		
Fuqua, S.A.W. ***Active Support***	NIH/NCI	5P50CA58183-04 SPORE in Breast Cancer, Project 2 Heat Shock Proteins and Drug Resistance	09/01/95-08/31/00	143,482

Fuqua, S.A.W. ***Active Support***	NIH/NCI	PO1CA30195-15 Medical Oncology Program Project; Therapeutic Research; Project 2; Molecular Variants of Estrogen and Progesterone Receptor in Clinical Breast Cancer	07/28/92-04/30/97	111,991
	NIH/NCI	5P50CA58183-04 SPORE in Breast Cancer, Project 1 Clinical Tamoxifen Resistance: Mechanisms and New Agents C. Kent Osborne, P.I.	09/01/95-08/31/00	174,867
	USAMRDS	DAMD17-94-J-4112 Fellowship to Study the Involvement of Heat Shock Proteins in Drug Resistance in Human Breast Cancer	09/01/94-08/31/97	61,661
	USAMRDC	DAMD17-95-1-5025 Fellowship to Identify New Mechanisms of Tamoxifen Resistance in Breast Cancer Patients	01/01/95-12/31/98	61,955
Pending Support	NIH/NCI	Hyper-sensitive Estrogen Receptor in Premalignant Breast	07/01/96-06/30/01	158,940
	ACS	Hyper-Sensitive Estrogen Receptor in Premalignant Breast	07/01/96-06/30/01	96,485
Klebe, R.J. ***Active Support***	San Antonio Breast Cancer SPORE Grant	Matrix Metalloproteinase Expression During Mammary Carcinoma Interactions with Stromal Cells	10/01/95-09/31/96	48,471
	NIH	R01DE08144-07 Initial Events in Bone and Tooth Morphogenesis	07/01/93-06/30/97	177,692
Pending Support	USAMRMC	Mammary Carcinoma Interactions with Stromal Cells	03/01/96-02/28/00	268,999

Leach, R.J. ***Active Support***	NIH	1P01HG00470 Saturation Mapping of Human Chromosome 3; Program Project Grant; Project 2	06/10/92-05/31/97	125,841
	NIH	1R01CA58127-01 Recessive Mutations in the Genesis of Prostate Cancer	09/30/92-09/29/96	21,966
	NIH	R29AR40689-01A1 Regulation of Osteoblast-Specific Gene Expression	07/01/91-06/30/96	59,417
	Private Donation	18q-Syndrome Research Study	01/01/94-Completion	500,000
Pending Support	NIH	Molecular and Neurodevelopmental Analysis of Aneusomy	04/01/96-03/31/02	1,101,136
Lee, E. Y.-H.P. ***Active Support***	NIH/NCI	1R29CA49649-08 Tumor Suppression Function of RB and p53 in the Mammary Gland	07/01/94-04/30/99	143,438
	NIH	HD30265 Mouse Models for Studies of the Retinoblastoma Gene	12/01/93-11/30/97	173,805
Pending Support	Texas Higher Education Coordinating Board	Molecular Mechanisms of the Neuronal Specific Function of the Retinoblastoma Gene	01/01/96-12-31-97	170,000
	Texas Higher Education Coordinating Board	Isolation of the Neuronal Progenitor Cells by Intervention of Neurogenesis and Differentiation	01/01/96-12-31-97	174,703
Masters, B.S.S. ***Active Support***	NIH	R37HL30050 Microsomal Electron Transport in Liver and Heart	04/01/88-03/31/97	150,302

Masters, B.S.S. ***Active Support***	Robert A. Welch Foundation	AQ1192 Structure-Function Relationships in the FAD- and FMN-Containing Enzymes, NADPH-Cytochrome P450 Reductase and Nitric Oxide Synthase	07/01/93-06/30/96	52,000
	NIH	R01GM31296-12 Prostaglandin 19- and 20-Hydroxylation by Cytochrome P450	06/01/94-05/31/97	144,721
Pending Support	NIH	Structural/Functional Modularity in Nitric Oxide Synthase	01/01/96-12/31/00	142,240
Mundy, G.R. ***Active Support***	NIH	RR01346 General Clinical Research Center	12/01/93-11/30/98	223,187
	NIH	PO1CA40035 Effects of Tumors on the Skeleton	06/01/95-05/31/99	953,137
	NIH	R37AR28149 The Monocyte-Macrophage System and Bone Resorption	07/01/91-06/30/96	190,170
	NIH	PO1AR39529 The Osteoclast and its Regulation-Administrative Core	04/01/93-03/31/97	45,630
	NIH	PO1AR39529 The Osteoclast and its Regulation; Project 1; Identification and Characterization of a Novel Peptide	04/01/93-03/31/97	136,880
	NIH	PO1AR39529 The Osteoclast and its Regulation; Laboratory Core	04/01/93-03/31/97	137,765

Mundy, G.R. ***Active Support***	OsteoSA	OsteoSA OsteoSA is a company whose mission is to identify agents which stimulate bone formation and may be developed as drugs in the treatment of bone loss.	01/01/95-12/31/95	2,064,000
Pending Support		none		
O'Connell, P. ***Active Support***	NIH	PO1HG00470-01 Saturation Mapping of Human Chromosome 3; Core A and Project 3	06/01/92-05/31/97	563,593
	NIH/NCI	P50CA58183-04 Translational Research in Breast Cancer--San Antonio, Project 4	09/01/95-08/31/99	134,052
	NIH/NCI	2P30CA54174 San Antonio Cancer Institute	08/19/94-07/31/98	61,644
	NIH	R01DK47482 NIDDM Susceptibility Genes in Mexican Americans	09/30/93-09/29/98	216,481
Pending Support	NIH/NCI	Molecular and Genetic Epidemiology of Gliomas	01/01/96-12/31/00	81,433
	NICHD	P01 Molecular and Neurodevelopmental Analysis of Aneusomey, Project 5	04/01/96-03/31/01	166,230
	NIH/NCI	PO1CA55261 Renewal Molecular and genetic Epidemiology of Gliomas	01/01/96-12/31/01	81,433
Seto, E. ***Active Support***	NIH/NCI	R01CA61257-01 Molecular Analysis of a Metallothionein Gene Initiator	12/15/93-11/30/97	99,078

Seto, E. ***Active Support***	Texas Higher Education Coordinating Board	#003659006 Molecular Analysis of a YY1-Binding Protein	01/01/94-12/31/95	84,949
Pending Support	NSF	MCB-9513165 Role of YAP1, A Mammalian Homolog of the Yeast Transcription Factor RPD3, in YY1 Mediated Transcriptional Activation and Repression	04/01/96-03/31/01	116,136
	NIH	A139473-01 Analysis of a Hepatitis B Virus X-Associated Protein	04/01/96-03/31/01	129,213
Sharp, Z.D. ***Pending Support***	NSF	Pit-1, Promoter Geometry and Transcription Activation in the Pituitary	10/01/95-09/30/98	139,844
	Texas Higher Education Coordinating Board	Regulated Overexpression of Growth Hormone by the Pituitary-Effects on Animal Growth	01/01/96-12/31/97	94,879
	March of Dimes	Subnuclear Partitioning of Developmental Control Transcription Factors	07/01/96-06/30/98	46,691
Tomkinson, A.E. ***Active Support***	NIH	R29GM47251-03 Cellular Functions of Eukaryotic DNA Ligases	08/01/93-07/31/98	77,579
	The Council for Tobacco Research	#3786 DNA Nucleotide Excision Repair in Eukaryotes	01/01/94-12/31/95	52,174
Pending Support	Texas Higher Education Coordinating Board	Identification and Characterization of Multiprotein Complexes Containing Mammalian DNA Ligase III	01/01/96-12/31/97	157,000

Von Hoff, D.D. ***Active Support***	NIH	1R03CA6268802 Gallium in Non-Hodgkin's Lymphoma in AIDS Patients	09/30/93-08/31/96	50,406
	National Foundation for Cancer Research	Intermediates in Gene Amplification	10/01/92-09/30/95	50,000
	NIH/NCI	1U01CA69853-01 Phase I Clinical Trials of Anticancer Agents	07/01/95-02/28/98	1,244,228
	NIH/NCI	RFACA9408NCDDG Telomere and Telomerase Interactive Agents	07/01/95-06/30/00	pending
	NIH/NCI	5R01CA56832-03 Elimination of Extrachromosomal DNA from Ovarian Cancer	071/17/92-06/30/96	86,733
Pending Support	Cap CURE Association for the Cure of Cancer of the Prostate	DNA Topoisomerase I - Targeted Therapy for Prostate Cancer	10/01/95 -	150,000
	USAMRMC	DNA Topoisomerase I - Targeted Therapy for Breast Cancer	06/01/96-05/31/00	800,000
Yee, D. ***Active Support***	NIH	R29CA52592-04 Growth Regulation of Cancer by IGF-I	07/01/90-06/30/96	56,863
	NIH	P50CA58183-02 Translational Research in Breast Cancer San Antonio; Project 2; Heat Shock Proteins and Drug Resistance	09/01/95-08/31/00	144,500
	NIH	P01CA30195-15 Medical Oncology Program Project; Therapeutic Research; Project 6; The IGF-System as a Potential Treatment Target in Breast Cancer	07/28/92-02/28/97	115,222

Yee, D. ***Active Support***	NIH	PO1CA30195-15 Medical Oncology Program Project; Therapeutic Research; Project 2; Molecular Variants of Estrogen and Progesterone Receptor in Clinical Breast Cancer	07/28/92-02/28/97	101,812
	PEW Scholars Program	Growth Regulation of Cancer by IGF-I	07/01/90-06/30/96	50,000
Pending Support	NIH	The IGF System Components in Breast Cancer Prognosis	04/01/96-03/31/00	131,164

4. Listing of Supported Trainees

Trainees receiving support from the Training Program in the Molecular Basis of Breast Cancer Research are selected from among entering first year students in the Molecular Medicine Ph.D. Graduate Program. In subsequent years of their training, they may be maintained on the Training Program, or transferred to other funding sources, depending on the nature of their research interests, and the availability of grant support. *There are currently a total of thirty students enrolled in the Molecular Medicine Ph.D. Program. Of those thirty students, only six are supported by the Training Program in the Molecular Basis of Breast Cancer. We apologize for the omission of these statistics which show that the Breast Cancer Training Program is not supporting the Molecular Medicine Ph.D. program more so than the Breast Cancer Program.* The following trainees were supported on the Breast Cancer Training Program.

1994-1995
Entering First Year Students

Christa Hargraves
Harold Pestana

Upper Level Students

Jim Fitzgerald
Zachary Mackey
Yuewei Qian
James Wang

1995-1996 Current Year
Entering First Year Students

Linda DeGraffenried
Jennifer Gooch
David Levin
Ernest Salcedo

Upper Level Students

Zachary Mackey
Harold Pestana

Disposition of Previous Trainees:

Jim Fitzgerald	currently funded by advisor's grant.
Christa Hargraves	left the program.
Zachary Mackey	continuing as an upper level student.
Harold Pestana	*withdrew from Graduate School after failure of comprehensive examinations.*
Yuewei Qian	currently funded by advisor's grant.
James Wang	currently funded by advisor's grant.

The 1995-1996 academic year marks the third full year of operation for the Molecular Medicine Ph.D. Program, and the second for the Training Program in the Molecular Basis of Breast Cancer Research. The availability of highly qualified applicants to the Molecular Medicine Program has proven to be excellent. 117 applications were received

for admission to the Fall 1995 entering class. Letters of acceptance were offered to 21 students, and 11 students began classes in August of 1995. The total number of students in the Molecular Medicine Ph.D. Program at all levels is 30, which includes 15 women, and 3 minorities (1 black, 2 Hispanic students). All three minority students are currently supported by the Training Program in the Molecular Basis of Breast Cancer Research.

5. Project Summaries of Upper Level Trainees

Students in the Molecular Medicine Program spend their first year doing rotations in different laboratories. Thus, they have not selected a laboratory for their thesis work. The project summaries in this section were written by upper level students in the training program.

Harold Pestana **Mentor: Z. Dave Sharp, Ph.D.**

Mr. Pestana worked in the laboratory of Dr. Sharp. The lab is interested in two members of the POU-homeodomain class of developmental regulatory proteins, Pit-1 and Oct-1. Pit-1 has been found to be necessary both *in vivo* and *in vitro,* for the activation of the Prolactin and Growth Hormone genes. For example, a homozygous mutation of this gene in the Snell dwarf mouse prevents DNA binding, which in turn leads to pituitary hypoplasia and severe deficiency in Growth Hormone, Prolactin Hormone, and Thyroid Stimulating Hormone.

Mr. Pestana withdrew from the Graduate School of Biomedical Sciences after failing comprehensive examinations.

Jim Fitzgerald **Mentor: Robert Christy, Ph.D.**

The Stearoyl-CoA Desaturase enzyme is a member of the cis-delta-9-desaturase complex, which catalyzes the conversion of Stearoyl-CoA (18:0) and Palmitoyl-CoA (16:0) to Oleoyl-CoA (18:1) and Palmitoleoyl-CoA (16:1) respectively. This is the rate limiting step in the production of membrane phospholipid synthesis and lipid storage. The transcriptional activity of the SCDI gene is regulated by dietary carbohydrates in liver, but not in adipose. I have examined the 5' flanking sequence of the SCDI gene for DNA/protein complexes which are altered in response to dietary manipulation, using nuclear extracts from mouse liver and 3T3-L1 adipocytes in culture. One such complex involves the CCAAT / Enhancer Binding Protein (C / EBP) family of transcription factors, while another represents a novel binding site / factor we have named SCD-Binding Protein (SCD/BP). Binding of dimerized C / EBP is constitutive in liver in response to dietary manipulation, however I have discovered that the composition of the isoforms bound to SCDI changes rapidly and transiently following refeeding. Binding of SCD/BP is positively correlated with SCDI gene expression. The binding complex is abolished by fasting, but reappears 2 hours following refeeding. In contrast, neither of these complexes is altered in 3T3-L1 adipocytes, by changes in the source or concentration of carbohydrates or by changes in serum concentration in the culture media. Investigation of the functional importance of the C/EBP and SCD/BP complexes in SCDI gene expression is currently under way using chimeric reporter gene constructs transfected into liver and adipose cells in vitro. These results suggest that the differential regulation of these transcription factors between liver and adipose may be responsible for the different responses of SCDI to dietary manipulation, and may have future implications on the management of obesity by dietary modification.

Yi-Chun James Wang **Mentor: Eduardo Montalvo, Ph.D.**

The BZLF1 gene of Epstein-Barr virus (EBV) is an important regulator of the virus latent / lytic cycle and expression of this gene product is sufficient to switch the virus from the latent state to lytic replication. My work has focused on identifying the anti-immunoglobulin response elements in the Epstein-Barr virus BZLF1 promoter and determining the cellular factors which regulate this promoter. By constructing various deletion in the promoter a region was mapped which overlapped with a previously identified TPA response element. I obtained point mutant of this region from Dr. Samuel Speck (Washington University, St. Louis) and was able to determine that the same site was in fact responsive to anti-immunoglobulin treatment of B cells.

Since then I have focused on identifying the cellular product(s) responsible for regulating this region. Although several members of the AP1 and the CREB family are able to transactivate the BZLF1 promoter in transient assays (measuring CAT activity), only one member of these proteins can reactivate latent EBV. The manuscript detailing this work is currently in preparation.

Yuewie Qian **Mentor: Eva Lee, Ph.D.**

The retinoblastoma protein (Rb) interacts with multiple cellular proteins that mediate its cellular function. I have identified nine polypeptides that bind to the T-binding domains of Rb using an Rb-affinity resin. RbAp48 and RbAp46 are quantitatively the major Rb-associated proteins purified by this approach. RbAp48 was characterized previously and was found to be related to *MSI1*, a negative regulator of Ras in the yeast *Saccharomyces cerevisiae*(Qian, Nature 364, 648-852, 1993). Recently, I have cloned and characterized RbAp46. RbAp46 shares 89.4% amino acid identity with RbAp48. The internal WD repeats, which are found in a growing number of eukaryotic proteins, are conserved between RbAp46 and RbAp48. Like RbAp48, RbAp46 forms a complex with Rb both *in vitro* and *in vivo*, and suppresses the heat-shock sensitivity of the yeast $RAS2^{Val19}$ strains. We have also isolated the murine cDNA homologues of RbAp48 and RbAp46. Although both mRNA can be detected in all mouse tissues, their mRNA levels vary dramatically between different tissues. No significant differences were observed in the expression patterns of these genes in most tissues except thymus, testis and ovary/uterus, in which two-fold differences were observed. Interestingly, the mouse and human RbAp48 amino-acid sequences are completely identical, and the mouse and human RbAp46 differ only by one conserved amino acid substitution. These results suggest that RbAp48 and RbAp46 may have shared as well as unique functions in the regulation of cell proliferation and differentiation.

Zachary Mackey **Mentor: Alan Tomkinson, Ph.D.**

In this project, we are studying a type of enzyme, DNA ligase that is required for maintaining the integrity of the genome. Mammalian cells contain three biochemically distinct species of DNA ligase. One of these enzymes, DNA ligase I, is required for DNA replication and also functions in DNA repair. We are seeking to determine the cellular functions of the other DNA ligases. DNA ligase II (70 kDa) and DNA ligase III (100 kDa) have been purified to homogeneity from bovine liver and testes, respectively. In the DNA joining reaction, these enzymes are more tolerant of mismatched DNA termini than DNA ligase I. Amino acid sequencing of peptides from DNA ligases II and III revealed

that these enzymes are probably encoded by the same gene and are more closely related to DNA ligase encoded by pox virusesthan to replicative DNA ligases, such as DNA ligase I. Using degenerate primers deduced from DNA ligase II peptides, a cDNA fragment was specifically amplified by the PCR. After confirming that when translated the DNA sequence of the PCR fragment encoded a polypeptide that was essentially identical with the peptide sequences obtained from DNA ligases II and III, the PCR fragment was used as a probe to isolate full-length human and murine cDNAs. The open reading frames encoded by these cDNAs encodes a 96 kDa polypeptide that contains sequences homologous with essentially all the peptides from bovine DNA ligases II and III. Our current working hypothesis is that this cDNA encodes DNA ligase III and that DNA ligase II is derived from DNA ligase III by a specific proteolytic processing mechanism.

Analysis of DNA ligase III expression by northern blotting demonstrated that this gene is highly expressed in the testes. DNA ligase I is also highly expressed in this tissue and we have shown that this gene is highly expressed in proliferating spermatogonia, consistent with DNA ligase I functioning in DNA replication. In contrast the high levels of DNA ligase III expression occur in primary spermatocytes undergoing meiotic recombination. It appears that DNA ligase III seals DNA strand breaks that have arisen as a consequence of meiotic recombination in germ cells whereas in somatic cells it functions to repair DNA strand breaks that occur following DNA damage.

6. Trainee Publications

Yue-Wei Qian, Yi-Chun J. Wang, Robert E. Hollingsworth, Jr., Diane Jones, Nicholas Ling, and Eva Y.-H. P. Lee (1993). A Retinoblastoma-Binding Protein Related to a Negative Regulator of Ras in Yeast. Nature 364, 648-652.

Carmel E. Hensey, Frank Hong, Tim Durfee, **Yue-Wei Qian**, Eva Y.-H. P. Lee, and Wen-Hwa Lee. (1994). Identification of Discrete Structural Domains in the Retinoblastoma Protein. The Journal of Biological Chemistry 269, 1380-1387.

Yue-Wei Qian and Eva Y.-H.P.Lee (1995). Dual retinoblastoma-binding proteins with properties related to a negative regulator of Ras in yeast. The Journal of Biological Chemistry (in press).

Wang, Y.-C.J., Burkhart, W.A., **Mackey, Z.B.,** Moyer, M.B., Ramos, W.,Husain, I., Chen, J., Besterman, J.M. and Tomkinson, A.E. Mammalian DNA ligase II is highly homologous with Vaccinia DNA ligase. Journal of Biological Chemistry 269, 31923-31928.(1994).

Husain, I., Tomkinson, A.E., Burkhart, W.A., Moyer, M .B., Ramos, W., **Mackey, Z.B.**, Besterman, J.M. and Chen, J. Purification and characterization of DNA ligase III from bovine testes. Journal of Biological Chemistry 270, 9683-9690 (1995)

Chen, J., Tomkinson, A.E., Ramos, W., **Mackey, Z.B.,** Danehower, S., Schultz, R.A., Besterman, J.M. and Husain, I. Mammalian DNA ligase III: Molecular cloning, chromosomal localization and involvement in meiotic recombination during spermatogenesis. Molec. Cell. Biol. 15, 5412-5422 (1995)

7. Changes to the Program Faculty:

Additions:

Alan E. Tomkinson, Ph.D. to Molecular Genetics SubProgram

Project Summary, Biographical Sketch, Research Support

Alan E. Tomkinson, Ph.D.
Assistant Professor, Institute of Biotechnology
Ph.D., The University of Newcastle upon Tyne, England

My laboratory is interested in the network of pathways which maintain genomic stability in eukaryotes. Defects in these pathways can lead to genetic instability, a decrease in cell viability and an increase in the frequency of carcinogenesis.

We have chosen to study eukaryotic DNA joining enzymes, DNA ligases, since mutations in the DNA ligase genes of prokaryotes and lower eukaryotes cause genetic instability. Mammalian cells contain at least three biochemically distinct DNA ligases. One of these enzymes, DNA ligase I, is required for DNA replication and also functions in DNA repair. Recently, we have isolated cDNAs encoding mouse and human DNA ligase III. This gene is ubiquitously expressed at low levels except in the testis, which contains about 10-fold higher levels of DNA ligase III mRNA. Within the testis, the highest levels of DNA ligase III expression are found in cells undergoing meiotic recombination, suggesting that DNA ligase III joins DNA strand breaks introduced as a consequence of meiotic recombination. The cellular functions of DNA ligase III in somatic and germ cells are being investigated.

The ultra-violet component of sunlight is a major environmental mutagen that causes skin cancer. This type of DNA damage is normally repaired by a nucleotide excision repair pathway that is conserved amongst eukaryotes. Patients with the inherited cancer-prone disease, xeroderma pigmentosum, are specifically defective in this repair pathway. Recently, we have characterized an endonuclease activity from yeast that is required for DNA lesion removal. Since this endonuclease has no apparent affinity for DNA damage, we are searching for interactions between this enzyme and other components of the repair pathway that will confer DNA damage-specificity on the endonuclease. The goals of these studies are to elucidate the molecular mechanisms of nucleotide excision repair.

8. Course Changes:

New Course:

Current Topics in Cancer Biology / Course Director: Eva Lee Ph.D.

A new course was established by Dr. Eva Lee covering Current Topics in Cancer Biology. The course is taught on an elective basis to upper level pre-doctoral and post-doctoral students. The course is presented in a combination of lecture and research article presentation format.

Regarding the new course, Current Topics in Cancer Biology. We respectfully submit that the criticism that there are only a few lectures devoted to breast cancer is again, perhaps, short sighted. In our opinion, breast cancer must be considered in the context of the general problem of cancer and biology. We submit that the important break throughs in breast cancer research will come from basic understandings in other tumorigenic or biological systems. One of the biggest mistakes a scientist can make is to work in the vacuum of a single system where outside ideas and interpretations can not be

productively incorporated into the research programs. It is this spirit in which this course was developed.

In addition, Drs. Kent Osborne, Doug Yee, and Suzanne Fuqua give annual presentations on the clinical relevance of basic research in breast cancer to the students supported by the Breast Cancer Program. Recently, we established a monthly Breast Cancer Research Conference that includes the students supported by the Training grant and a cadre of breast cancer researchers working in the San Antonio area. Each month the principal investigators will present an informal one hour talk about their work and their vision of its future. The students in the training program will also sponsor, in conjunction with Molecular Medicine, a distinguished breast cancer scientist as a seminar speaker.

A report on the initiatives of breast cancer research was inadvertently omitted from the progress report. However we are pleased to report, that the seminar in the fall term of 1994-95 included two distinguished speakers, Dr. Marc Lippman, whose seminar was entitled "Regulation of angiogenesis in human breast cancer," and Dr. Bert O'Malley, whose seminar was entitled "Activation of steroid receptor superfamily members and the uses for gene therapy."

During the first semester of 1995-96, Molecular Medicine hosted a minisymposium on Signal Transduction and Cell Cycle. The keynote speaker was Dr. Tony Hunter whose topic was "Cell cycle regulation by protein phosphorylation." The other speakers and their topics were: Dr. Yue Xiong, "CDK inhibitors: Their function in tumor growth suppression and cell differentiation;" Dr. Ted Weinert, "Yeast cell cycle checkpoints and lesion processing model;" Dr. Paul Russell, "Stressing the cell cycle: MAP kinase pathway links G2/M control with cellular signals."

Attendance at the annual San Antonio Breast Cancer Symposium is required by all of the students supported by the Training Program in the Molecular Basis of Breast Cancer. Dr. Bert O'Malley was the William L. McGuire Lecturer at this year's symposium.

Student progress reports are a requirement of the breast cancer training program, in conjunction with the Molecular Medicine Program. This is attended by the faculty of Molecular Medicine and is the forum where students get feedback from other scientists on their work.

The last point of the progress report concerns a failure to highlight any strong association between clinical activities and the Breast Cancer Program. Graduate students are not permitted to participate in clinical duties. To circumvent this problem, the logistics for a forum to inform breast cancer program trainees about the current treatment protocols and for the students to communicate to the physicians information about their research is currently being explored. Until this is organized, we will require that students attend a monthly Medical Oncology research conference. It is also pointed out that each breast cancer-supported graduate student must have a dissertation advisory committee with at least one breast cancer clinician.